REMARKS/ARGUMENTS

After amendment, the pending claims are 1-11, 13-17, 28-37, and 39-44. Claims 1 and 43 are amended to clarify the invention, namely to specify that the desired amino acid substitution which creates the mutant cholera holotoxin replaces the glutamic acid which naturally occurs at position 29 of the A subunit of the ***wild-type*** cholera holotoxin with an amino acid other than aspartic acid. Claim 3 is rewritten in independent form. Claims 15, 16, 29, 41, and 42 are amended to clarify that the amino acid positions refer to wild-type CT amino acids positions and/or to correct minor clerical errors. New Claim 44 is an independent claim based on Claim 3 and Claim 29. Support for these amendments is found in throughout the specification. No new matter is added by these amendments.

Allowable Subject Matter

Claims 3 and 29 are objected to as being dependent upon a rejected base claim.

Applicant requests reconsideration and withdrawal of this objection in view of the above-noted and below-discussed claim amendments. In view of these amendments which clarify the invention, Applicants submit that all claims, including claims 3 and 29 are in condition for allowance.

35 USC § 102(b) Rejection

(i) *Claims 1-2, 4, 6-8, 11, 13-17, 28, 30, 32-34, 38, and 39-43 are rejected under 35 USC § 102(b) over Rappuoli et al. (International Patent Publication No. WO 95/17211) as evidenced by Zhang et al. (J. Mol. Biol., 251: 564, 1994).*

The Examiner asserted that Rappuoli discusses a mutant cholera holotoxin where the mutation is a deletion at position 7, which would result in a substitution of tyrosine at position 29 as evidenced by Zhang which asserts that upon deletion of position 7, the tyrosine at position 30 would become position 29.

Applicants respectfully request reconsideration and withdrawal of this rejection in view of the above claim amendments and following remarks. Specifically, Applicants

have amended the independent claims and various dependent claims to clarify that the amino acid residues discussed are those of the wild-type CT sequence. Note that, as mentioned in the prior response, all of the known variants of CT have a glutamic acid residue at their naturally-occurring (or wild-type) residue position 29. Specifically, the above amendments clarify that Applicants' mutant CTs contain an amino acid (other than Asp) that replaces the naturally-occurring Glu that occurs at ***wild-type CT-A subunit position 29*** – **not** at the 29th amino acid position of any resulting mutant.

The documents cited by the examiner do not teach or suggest a mutant CT as described by the amended claims. Zhang provides the sequences, and comparison thereof, of wild-type cholera toxin (CT) and wild-type *E. coli* heat labile toxin (LT) in Fig. 1 of page 564. Zhang indicates that at the wild-type position 29 of both toxins, the naturally-occurring amino acid is a glutamic acid. Zhang does not discuss any substitutions of the amino acid at the wild-type position 29. Zhang provides the wild-type sequences of CT and LT.

Rappuoli refers to immunogenic compositions comprising an immunologically effective amount of an antigen and a mucosal adjuvant, which latter component is generally a detoxified mutant of a bacterial toxin, such as CT and LT, having one or more amino acid additions, deletions or substitutions in the A subunit of the holotoxin. However, Rappuoli specifically teaches an LT mutant possessing an Arg7 to Lys7 substitution and no specific CT mutant. Nowhere in Rappuoli is there any reference to a mutant cholera toxin subunit A which replaces the ***naturally-occurring glutamic acid*** that occurs ***at wild-type CT-A position 29***. In fact, Rappuoli does not teach or suggest the motivation to select amino acid position 29 for modification of any of the bacterial toxins.

Further, the fact that one could arbitrarily delete any one of the wild-type amino acids at positions 1-29 of CT with the simple knowledge that the amino acid at position 30 would become amino acid 29 does not teach Applicants' claimed invention. As Zhang teaches, the sequence of CT-A, both wild-type and variants thereof, are known in the art. While the variants of CT-A have sequence variations, all of these variants

contain a naturally-occurring glutamic acid at ***wild-type*** position 29. In the present invention, it was Applicants' substitution of that naturally occurring glutamic acid ***at wild-type CT-A position 29*** with another amino acid in any of the CT-A variants that results in mutant cholera holotoxins and their use in antigenic compositions.

If one were to arbitrarily delete an amino acid from any of positions 1-29 of the A subunit of the cholera holotoxin sequence, the naturally-occurring glutamic acid at wild-type position 29 would be shifted instead of replaced with another amino acid. This is contrary to Applicant's invention, which requires that the glutamic acid that occurs naturally at wild-type position 29 be replaced, not simply moved to a different position in the sequence by virtue of an amino acid deletion of any of the amino acid residues in wild-type positions 1-28. Therefore, Rappuoli does not teach or suggest the present invention, as presented in Applicants' claims.

In view of the pending claims which require a substitution ***at wild-type CT-A*** position 29, this rejection may be properly withdrawn.

(ii) *Claims 1, 2, and 13 are rejected under 35 USC § 102(b) over Glineur et al. (Infection and Immunity, 62(10):4176, 1994).*

The Examiner asserted that Glineur discloses an antigenic composition that comprises a mutant holotoxin of cholera toxin with a tyrosine substituted at p position 29.

Applicants respectfully request reconsideration and withdrawal of this rejection in view of the following remarks.

Glineur refers to a study of the importance of ADP-ribosylation in the morphological changes of rat PC12 cells induced by cholera toxin. Glineur states and suggests nothing at all with respect to use of cholera toxin mutants as ***adjuvants*** for other antigens. Glineur discusses only two mutations of cholera toxin subunit A:

(1) a cholera toxin subunit A having an amino acid ***deletion*** at amino acid position 29; and

(2) a cholera toxin subunit A in which the naturally-occurring glutamic acid at amino acid position 29 with the ***most conservative*** amino acid replacement for glutamic acid, i.e., aspartic acid (E29D).

Glineur noted that mutation (1) extinguished all toxicity and enzymatic activity of cholera toxin. Mutation (2), i.e., replacement with the positively charged, conservative aspartic acid, had "no significant effect" on the enzymatic activity or toxicity of cholera toxin (page 4182, col. 2, last two paragraphs). Glineur does not teach that ***any*** amino acid replacement for amino acid position 29 would provide a cholera toxin mutant with the properties necessary for use as an adjuvant. In fact, since neither of modifications (1) or (2) had a significant effect on the enzymatic activity of cholera toxin, Glineur effectively ***teaches away*** from the antigenic compositions of the present invention. It is only Applicants' disclosure that provides the suggestion and support for successful use of a non-conservative amino acid replacement of E29 to create a mutant cholera toxin as an adjuvant.

For the reasons stated above with respect to Rappuoli, the deletion of any of the amino acids prior to the naturally-occurring Glu at wt CT-A position 29 would shift the specified wild-type Glu residue to another position in the sequence. However, Applicants' invention is not such a mutant. The pending claims require an amino acid substitution ***other than Asp*** in place of the naturally-occurring Glu that is located at position 29 in ***wild-type CT-A***. Glineur in no way teaches or suggests this requirement of the pending claims. Thus, Glineur does not anticipate the invention of the amended claims. This rejection may be properly withdrawn.

Additional Documents

The Examiner asserted that Feil et al. (Molecular Microbiol., 20(4):823-832, 1996) fails to show site directed mutagenesis of positions 47-53 of heat labile enterotoxin.

The Examiner also asserted that Gu et al. (US Patent No. 6,685,949) show the utilization of a CRM molecule as a carrier for a detoxified lipopolysaccharide in a method of inducing an immune response.

The Examiner further asserted that Vadheim et al. (Microbiol., Pathogenesis, 17: 339, 1994) discusses a composition comprising a mutant recombinant cholera

toxin with a methionine at position 29, due to the deletion of aa 6-13, thus substituted the methionine at position 38 for the glutamic acid at position 20. New position 29 after deletion of aa 6-13 in subunit A, resulted in the substituted of methionine at position 20.

Applicants assert that Feil, Gu, and Vadheim do not teach or suggest Applicants' present invention for the same reasons as stated above. The mere shifting of the naturally-occurring Glu at wt CT-A position 29 to another position by virtue of deletion of another amino acid of CT does not effect replacement of that Glu residue and is ***not*** Applicants' claimed invention.

In view of the above amendments and remarks, Applicants request that all claims be found in condition for allowance and proceed to issuance in due course.

The Director is hereby authorized to charge any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees to our Deposit Account Number 08-3040.

Respectfully submitted,

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